Functional Characterization of Amphipathic alpha-Helix in the Osmoregulatory ABC Transporter OpuA

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Supplementary Materials

Functional characterization of amphipathic α-helix in the osmoregulatory ABC transporter OpuA

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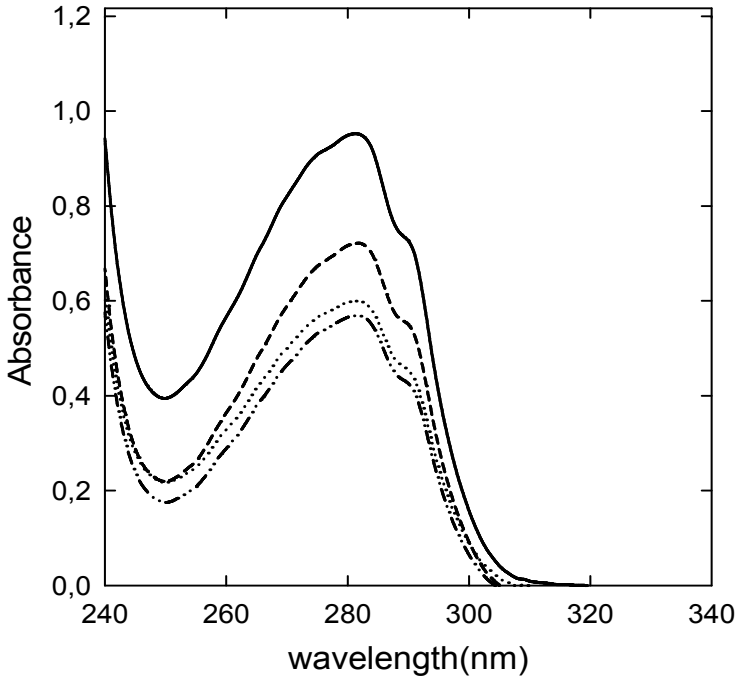
Mutant	Modification	Primers (5'- 3')
T23C	T23C	**C**CATAGCAAACTGGGTGTCATCGGCAACAGATTGGATTTGTAGTACTTTTAGTT CAGGATTT**GACGT** **C**AAATCCTGAACTAAAAGTACTACAAATCCAATCTGTTGCCGATGACACCCAGT TTGCTATG**GGTAC**
S24C	S24C	**C**CATAGCAAACTGGGTGTCATCGGCAACAGATTGGATTACTTGTACTTTTAGTTC AGGATTT**GACGT** **C**AAATCCTGAACTAAAAGTACAAGTAATCCAATCTGTTGCCGATGACACCCAGTT TGCTATG**GGTAC**
T25C	T425C	**C**CATAGCAAACTGGGTGTCATCGGCAACAGATTGGATTACTAGTTGTTTTAGTTC AGGATTT**GACGT** **C**AAATCCTGAACTAAAACAACTAGTAATCCAATCTGTTGCCGATGACACCCAGTT TGCTATG**GGTAC**
Scramble	N13W, W14N, S17A, A18S, I22T, T23I , F26S, S27F	***C**CATAGCATGGAATGTGTCAGCTTCAACAGATTGGACAATTAGTACTTCATTTTCA GGATTT**GACGT** **C**AAATCCTGAAAATGAAGTACTAATTGTCCAATCTGTTGAAGCTGACACATTCCA TGCTATG**GGTAC***
Pltp1	FKKVYDFLSTFI TSGMRF	**C**CTTTAAAAAAGTTTATGATTTTTTATCAACATTTATTACTAGTGGTATGCGTTTTG GATTT**GACGT** **C**AAATCCAAAACGCATACCACTAGTAATAAATGTTGATAAAAAATCATAAACTTTT TTAAAG**GGTAC**
Pltp2	FANVYDFLSTFI TSGMSF	**C**CTTTGCTAATGTTTATGATTTTTTATCAACATTTATTACTAGTGGTATGTCATTTG GATTT**GACGT** **C**AAATCCAAATGACATACCACTAGTAATAAATGTTGATAAAAAATCATAAACATTA GCAAAG**GGTAC**
Pltp3	FANVYSFLSDFI TSGMSF	**C**CTTTGCTAATGTTTATTCATTTTTATCAGATTTTATTACTAGTGGTATGTCATTTG GATTT**GACGT** **C**AAATCCAAATGACATACGACTAGTAATAAAATCTGATAAAAATGAATAAACATT AGCAAAG**GGTAC**
3K	S17K, S24K, S28K	**C**CATAGCAAACTGGGTGTCAAAAGCAACAGATTGGATTACTAAAACTTTTAGTA AAGGATTT**GACGT** **C**AAATCCTTTACTAAAAGTTTTAGTAATCCAATCTGTTGCTTTTGACACCCAGT TTGCTATG**GGTAC**
A	SGTVLMNAGIT GAL	GCACTG**GGCGCGCTA**ACTGCTGTTCCATTCTGGTTGATG (fwd A) CAGTCG**TAGCGCGCC**TGTAATACC**AGC**GTTCATCAATACCGTTCC
AA	SGTVLMNAAGI TGAL	GCACTG**GGCGCGCTA**ACTGCTGTTCCATTCTGGTTGATG (fwd A) CAGTCG**TAGCGCGCC**TGTAATACC**AGCAGC**GTTCATCAATACCGTTCC
AAA	SGTVLMNAAA GITGAL	GCACTG**GGCGCGCTA**ACTGCTGTTCCATTCTGGTTGATG (fwd A) CAGTCG**TAGCGCGCC**TGTAATACC**AGCAGCAGC**GTTCATCAATACCGTTCC
AAAA	GTVLMNAAAA GITGAL	GCACTG**GGCGCGCTA**ACTGCTGTTCCATTCTGGTTGATG (fwd A) CAGTCG**TAGCGCGCC**TGTAATACC**AGCAGCAGCAGC**GTTCATCAATACCGTTCC

STable1: Primers used for construction of the mutants. Restriction sites are indicated in bold and underlined. Nucleotides different from wild type OpuA are indicated by red color.

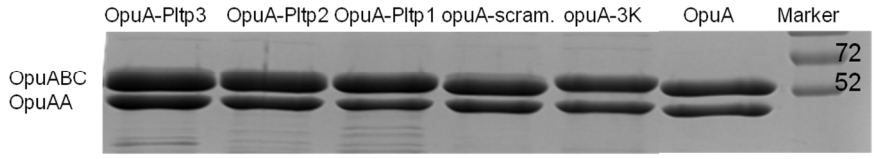
Protein	Labeling efficiency (%)	ATPase activity (mmol/min*mg of OpuA)
WT	-	3.5 ±0.1
S24C-5FM	80	3.74 ±0.04
V295C-5FM	89	3.1 ±0.1
nanodisc wo OpuA	-	0.26 ±0.01

STable 2. Labeling efficiency and ATPase activity of Cys mutants.
The labeling efficiency was determined from the molar ratio of OpuA and F5M calculated from the absorbance at 280 nm and 495 nm and the molar extinction coefficient as described in Materials and methods. The ATPase assay was performed in 50 mM KPi, pH 7.0, plus 300 mM KCl as described in Materials and methods.

A



B



SFigure 1: Purification and stability of wildtype OpuA and mutant derivatives.
(A): UV spectra of Ni-Sepharose purified proteins: wildtype OpuA (solid black line), OpuA3K (dashed and dotted line), OpuAPltp1(dotted line) and OpuAscramble(dashed lines).
(B): SDS-PAGE of purified proteins. The proteins were separated on a 12.5% polyacrylamide gel and stained with Coomassie brilliant blue. 5 µg of protein was loaded per lane.